8-2018

Evaluation of blight resistance in chestnut f2 half-sibling and full-sibling families via small stem assay

Kevin Gentner

University of Tennessee at Chattanooga, ngm928@mocs.utc.edu

Evaluation of Blight Resistance in Chestnut F2 Half-sibling and Full-sibling Families via Small Stem Assay

Kevin Gentner

Departmental Honors Thesis

The University of Tennessee at Chattanooga

Department of Biology, Geology, and Environmental Sciences

Examination Date: 10 April 2018

Dr. J. Hill Craddock

UC Foundation Davenport Professor of Biology, Geology, Environmental Science

Thesis Director

Dr. DeAnna Beasley

Assistant Professor of Biology, Geology, Environmental Science

Department Examiner

Dr. Andrew Bailey

UC Foundation Associate Professor of Health and Human Performance

Examiner

Table of Contents Page

Abstract

Evaluation of Blight Resistance in Chestnut F2 Half-sibling and Full-sibling Families via Small Stem Assay

Kevin Gentner

In 1904, *Cryphonectria parasitica*, the causal agent for chestnut blight, was imported into North America on chestnut nursery stock from China. Fifty-five years later, nearly all full-grown wild American chestnut trees (*Castanea dentata*) were dead. A century of work has been put into restoring the American chestnut to its rightful place among the forest canopy. Since the 1980s, The American Chestnut Foundation has pursued backcross breeding to introgress blight resistance into *C. dentata* from the resistant Chinese species (*C. mollissima*), and has used progeny testing to make predictions about parental resistance in B3F2 chestnuts. We performed a small stem assay on first year and one-year-old seedlings to measure variation of resistance within and among hybrid progeny of five cross types (F1, B1, BB1, F2, B3F2), and to eliminate blight-susceptible seedlings before they are planted in the field. We inoculated over 1,100 seedlings with *C. parasitica*. The small stem assay did not prove to be a reliable method of differentiating between generational resistance. Although there was significant difference in canker length in the American and Chinese control groups, hybrid crosses and the American control did not exhibit canker length averages inferred from their generation types, and all of the canker length means of the interspecific crosses didn't significantly differ from the American control, as shown by Duncan's multiple range test.

Acknowledgements

Thank you, Dr. Hill Craddock, for inspiring me inside and outside of the classroom; Thank you Taylor Perkins, for curing my aversion for data analysis; Thank you Paula Zannina, for keeping us on track and staying positive; Thank you Kirsten Hein and Trent Deason; Thank you members of the Fortwood greenhouse crew and all volunteers across TACF!

List of Tables and Figures

1. Introduction/Literature Review:

1.1 *Castanea dentata*

At the turn of the 20th century, the American chestnut, *Castanea dentata* (Marsh.) Borkh. (Fagaceae) was found throughout the Appalachian Mountains, its native range extending from central Alabama to southern Ontario (Russell, 1987). This canopy-dominant tree was considered a foundation species, as many species of animals utilized the tree's sweet nuts as a source of food (Ellison et al, 2005). It was also an excellent source of lumber due to its tall, straight growth and tannins which conferred resistance to decay (Anagnostakis, 1987; Anagnostakis, 2001). Then, in 1904, *C. dentata* trees in the New York Zoological Garden were observed with bright-orange cankers on their bark, a disease that would later be identified as chestnut blight (Merkel, 1905).

Cryphonectria parasitica (Murr.) Barr (Cryphonectriaceae), the causal agent of this bark canker disease, is an ascomycete fungus that is suspected to have entered North America on nursery stock from Asia, where it is endemic (Anagnostakis, 1987). On Chinese chestnut (*Castanea mollissima* Blume), and Japanese chestnut (*C. crenata* Sieb. & Zucc.), which have moderately to highly resistance to the disease, the fungus invades the bark causing small lesions and swelling. On *C. dentata*, the fungus destroys the vascular cambium, creating a pimpled, pustular canker that eventually causes the distal part of the branch to die, resulting in a characteristic wilting symptom (Anderson, 1914). Attempts at quarantining the fungus were unsuccessful, and approximately 45 years after entering the United States, the fungus had spread via airborne spores across *C. dentata*'s native range (Anagnostakis, 2001). By 1960, nearly all full grown American chestnuts were dead (Anagnostakis, 1987).

While some believe the relative abundance of *C.dentata* throughout the eastern U.S. has been exaggerated, the American chestnut is still thought of as an invaluable tree, whose loss has been a detriment to both ecological systems and the U.S. economy (Faison and Foster 2014). Recognizing this ecological disaster, Charles Burnham proposed backcrossing Chinese chestnut (*Castanea mollissima* Blume) to the American chestnut in order to introgress resistance into *C. dentata* (Burnham et al., 1981). The American Chestnut Foundation (TACF) was founded in 1983 as a non-profit to help fund work on Burnham's proposal (Hebard, 2006).

1.2. Backcross Breeding

Backcrossing is a method used throughout commercial agriculture and has been implemented in many cash crops, including corn (Harlan et Pope, 1922). *C. dentata* and *C. mollissima* are homologous, meaning backcrosses between these species is possible (Jaynes, 1962). Burnham and others theorized that after crossing these species and creating a F1 hybrid, 3 backcrosses to American would be sufficient to conserve important American chestnut adaptive characteristics (stature, cold hardiness, rot-resistant wood), while still retaining the ancestral Chinese alleles for resistance, so long as at each generation the progeny are selected for blight resistance (Burnham et al, 1981).

The third backcross generation are on average fifteen-sixteenths American (about 94 percent) even without selection for American morphological traits (Burnham, 1987). However, the best of the B3 trees will be only of intermediate resistance-- one parent in each backcross is a fully-susceptible American chestnut. In order to recover full resistance, selected B3 trees are intercrossed, resulting in a B3F2 generation. This B3F2 population is expected to yield a wide distribution of phenotypes ranging from high

susceptibility to high resistance (Burnham et al., 1981). It is in this generation that chestnut hybrids may inherit both alleles for blight resistance at each locus, creating the tree that will be reintroduced into the Appalachian Mountains. This method of backcrossing has been implemented by all 16 state chapters of TACF and has been expanded through advances in molecular genetics (Burnham, 1988).

There are 4 families in my experiment in what is known as the "Better Backcross," generation (BB1). BB1s are the progeny of a straight F1 crossed with a selected B3 hybrid (instead of *C. dentata*). This is advantageous because the B3 tree carries the resistance alleles inherited from its *C. mollissima* ancestor, which increases the average resistance of the progeny when compared to a normal B1 cross (Hebard, 2006).

Research using dominant anonymous markers lead researchers to propose a 3 gene model for blight resistance that accounted for 70 percent of the phenotypic variance for blight resistance (Kubisiak et al, 1997). Given this model, in an F2 family, the odds of an individual keeping the alleles for resistance at all loci is less than 1 in 64 plants (Kubisiak et al, 1997). Because of this, I am working with over 400 B3F2 trees in my sample in order to increase the odds of recovering a highly resistant B3F2 hybrid.

Although much of what we know about backcross breeding is based on fundamental plant breeding methods, there are some precautions to keep in mind when comparing my data set to Mendel's pea plants. For one, Mendel underwent several generations of inbreeding among the different pea plants he was studying. This was to guarantee homozygosity of each cross before doing any intercrosses between families. The same cannot be said for the controls in my experiment, i.e. although the American type is susceptible, it may not be homozygous recessive for resistance at all alleles. In

fact, low levels of resistance have been shown in wild-type American chestnuts (Griffin et al, 1983).

Mendel was also studying qualitative traits (wrinkled vs smooth, purple vs white), whereas I'm studying a quantitative trait (canker length). Therefore, it is harder to predict the genotype of an individual when analyzing a single phenotypic response. According to the phytopathology disease triangle, much of a plant's ability to fight an infection by a pathogen hinges on environmental factors (Stevens, 1960). At the B3F2 generation, the ideal tree with Chinese resistance to *C. parasitica* and the American form may be overlooked because the plant was growing in poor conditions or was potentially infected by some other pathogen (Stevens, 1960). To circumvent this, TACF has adopted a method of determining blight resistance known as progeny testing.

1.3. Determining Blight Resistance

Progeny testing is used in order to assess a B3F2 hybrid's blight resistance, where the hybrid is crossed with several other trees of differing families. The seed progeny (B3F3) produced are grown up and the average canker length across these plants is used to estimate the level of blight resistance of the parent tree. Seedlings from my trial will be planted in a backcross orchard, and when they are old enough to flower and produce seeds, progeny testing will be used to identify trees with high amounts of resistance.

Traditionally, to assess an individual tree's blight resistance using progeny testing, a standard assay would require planting hundreds of seeds of that fully-grown tree's progeny in an orchard. Three to five years later, those trees which survived are inoculated with *C. parasitica* and observations are made of the parent based on the

progeny's observed resistance. Many years of work and resources may determine that a tree only has moderate amounts of resistance. In my experiment, we conducted a small stem assay, where seedlings are inoculated in their first growing season, roughly 5-6 months old. The small stem assay if proven to be effective would shorten the time needed to determine the resistance of a family and allow for more hybrids to be screened each year. It could include techniques such as DNA marker-assisted selection and or resistance assays on younger, container-grown plants in a greenhouse/nursery environment to pre-screen progeny before orchard establishment (Westbrook and Jarret, 2018). The small stem assay also serves to cull the worst growing seedlings by subjecting them both to nursery growth conditions and a blight inoculation, allowing the best seedlings from each generation will be identified and planted in an orchard for further analysis.

Although the entire genomes of many cash crops (beet, cabbage, maize) are fully sequenced, much of the *C. dentata* genome and specific genes that confer disease resistance are still unknown (Kubisiak, 2013). The construction of gene maps for *C. dentata* will lead to more Quantitative Trait Loci (QTL) identification for marker assisted selection against blight susceptibility (Kubisiak, 1997). This in combination with progeny testing could expedite the process of selecting for resistance even further. Genetic analysis of *C. dentata* is being pursued by other plant geneticists (Kubisiak, 2013; Kubisiak, 1997; Fang, 2013; Santos, 2017).

2. Hypothesis:

If the Small Stem Assay correlates well with orchard-planted assays, The American and Chinese controls will exhibit appreciable differences in resistance to chestnut blight. Due to the heterozygosity of the F1 generation and because blight

resistance follows an incomplete dominance heritability (Burnham, 1988; Steiner et al, 2016), the mean canker length of the F1 cross type should be about the average between the mean canker length values of the two control groups. The F2 and B3F2 segregating populations should have the highest variation in canker length, due to the variation in the inheritance of *C. mollissima* inherited alleles for resistance. All interspecific hybrid crosses should exhibit an average canker length in between the two controls. If all these hypotheses hold true, then the SSA will have been a good indicator of resistance.

3. Materials and Methods:

3.1. Growing the Seedlings

Seeds from 38 genetic families of 5 generations (F1, B1, BB1, F2, B3F2,) and Chinese and American (CH and AM) species were obtained from contributing scientists of TACF representing more than 20 years of work breeding and selecting for blight resistance. We planted the seeds in January and February 2017 in the UTC Fortwood Greenhouse in Chattanooga, Tennessee (Table 1). A commercial potting medium (Sungrow Horticulture) was used, consisting of 50-60% composted pine bark, Canadian Sphagnum peat moss, perlite, vermiculite, and dolomitic lime. Seedling pots were top-dressed with a slow-release, encapsulated plant food (Osmocote). Seeds from some large families were planted both in 40 cubic inch containers (Stuewe & Son's D40) and 2-gallon containers (Stuewe & Sons TP812). The remainder were planted in D40 containers.

Pedigree	Generation	Famiy Code	Source of Resistance	Pedigree of Mother	Pedigree of Father
Haun (AM)	AM	1	ø	American	American
CAT 33 x Pryor 180 (AM)	AM	2	ø	American	American
CAT-275 x Neel 4-195 (B1)	B1	3	Amy	American	2004 TN-BF1-E10 x Amy
CAT-273 x TTU A29 (B1)	B1	4	Gideon	American	2004 TNCLA1 x Gideon
TN-TTU-A34 x NCDOT (B1)	B1	5	Gideon	2004 TNCLA1 x Gideon	American
CG61 x Pryor 180 (B1 NK2)	B1	6	Nanking	Ted Farmer B x GR 199 'Nanking'	American
CG61 x NCDOT (B1 NK4)	B1	7	Nanking	Ted Farmer B x GR 199 'Nanking'	American
CAT-273 x TN-CN 9-153 (B1)	B1	8	Chinese	American	Whiteside x opCh
TN-SM1-Q/S58 x OP (B3F2)	B3F2	9	Clapper	2002 TNBLO1 x GL103	OP
TN-SM2-C37 x OP (B3F2)	B3F2	10	Clapper	2007 AG387 x TNMAC2	OP
TN-SM2-E29 x OP (B3F2)	B3F2	11	Clapper	2006TNMON5 x HE416	OP
TN-SM2-G27 x OP (B3F2)	B3F2	12	Clapper	2006 TNMON4 x IL332	OP
TN-SM2-G44 x OP (B3F2)	B3F2	13	Clapper	2007 VA89 x TNJAC5	OP
TN-SM2-G-56 x OP	B3F2	14	Clapper	2007 VA89 x TNJAC5	OP
TN-SM2-H37 x OP	B3F2	15	Clapper	2007 GL367 x TNGSMNP1	OP
TN-SM2-H56 x OP	B3F2	16	Clapper	2007 VA89 x TNJAC5	OP
TN-SM2-I28 x OP	B3F2	17	Clapper	2007 NCGRA1 x GL96	OP
TN-SM2-I31 x OP	B3F2	18	Clapper	2007 NCGRA1 x GL96	OP
TN-SM2-I33 x OP	B3F2	19	Clapper	2007 NCGRA1 x GL96	OP
TN-SM2-J28 x OP	B3F2	20	Clapper	2007 TNMON8 x GR210	OP
TN-SM2-J39 x OP	B3F2	21	Clapper	2007 TNMON8 x GR210	OP
TN-TTU-M13 x OP	B3F2	22	Graves	2004 TNCLA2 x AB248	OP
TN-TTU-C27 x TN-TTU-A30	B3F2	23	Clapper and Gideon	2004 TNSUM1 x VA89	2004 TNCLA1 x Gideon
TN-TTU-E6 x Neel 5-275	BB1	24	Clapper, Meiling and Lindstrom 67	2004 TNSUM1 x VA89	2004 TN-BF3-L10 [1996 TN-BF1-D5 (American) x AP1-1 (Meiling x

					American)] x Lindstrom 67
TN-TTU-E6 x TN-TTU-A30	BB1	25	Clapper and Gideon	2004 TNSUM1 x VA89	2004 TNCLA1 x Gideon
TN-TTU-M10 x A30	BB1	26	Graves and Gideon	2004 TNCLA2 x AB248	2004 TNCLA1 x Gideon
TN-TTU-M13 x TN-TTU-A30	BB1	27	Graves and Gideon	2004 TNCLA2 x AB248	2004 TNCLA1 x Gideon
Smith Farm Chinese	CH	28	Chinese	Chinese	Chinese
Princeton MA Chinese	CH	29	Chinese	Chinese	Chinese
NCBUN10 x CC-PR05-4-42	F1	30	Chinese	Chinese	American
TNCOC1 x Nanking	F1	31	Chinese	American	Chinese
WWC67 x OP (NK5)	F2	32	Nanking	GR119 'Nanking' x KH2UU	GR119 'Nanking' x KH2UU
WWC70 x OP (NK6)	F2	33	Nanking	GR119 'Nanking' x KH2UU	GR119 'Nanking' x KH2UU
TN-SM1-C59 x OP	F2	34	Ginyose	2008 TNMON7 x Ginyose	OP
TN-SM1-D41 x OP	F2	35	Sleeping Giant	2005 KYADA1 x Sleeping Giant	OP
TN-TTU-A10 x OP	F2	36	Gideon	2004 TNCLA1 x Gideon	OP
NJ Paris F1	F1	37	Paris	Paris AM	Paris opCH
Greg Miller Chinese	CH	38	Chinese	Greg MIller Chinese Mix	opCH

Table 1. The list of 38 families that entered the trial, including generation, source of resistance, pedigree of mother, and pedigree of father; Trees that are open pollinated are designated (OP)

The seedlings were moved out of the greenhouse once the weather was warm enough to support healthy growth. The seedlings in the 2-gallon pots were watered via drip irrigation, while the D-40 container seedlings were watered by hand. At inoculation, each plant was given a unique identification number and tag. The seedlings were completely randomized across the plot to account for environmental differences.

Due to communication errors in planting, all of the families planted in 2-gallon containers did not have high numbers of individuals planted in D40s, meaning we were unable to observe differences in resistance of a single family between D40 and 2-gallon containers. Due to time constraints, generational comparisons will be made among those

trees planted in 2-gallons at a later date.

3.2. Inoculation

Isolate SG2-3, a weakly pathogenic strain of *Cryphonectria parasitica*, was obtained from the TACF lab in Meadowview, Virginia. The inoculum was prepared on potato dextrose agar (PDA) Petri dishes (Anagnostakis, 1977). Of the 1,299 seeds planted, 1,132 of the population were deemed okay to inoculate. Each seedling had to meet minimum growth requirements of at least 25 cm tall, and greater than 3 mm in diameter at 10 cm above the root collar. If the seedling was infected by other plant diseases that would affect its ability to combat the chestnut blight, it was also removed from the trial.

The seedlings were inoculated on the 8th and 9th of July 2017 (approximately 4-5 months after planting) with the help of the Fortwood Greenhouse Crew and several volunteers. The immature bark of each seedling was sliced open roughly 10 cm above the root collar using a nitpicker (this distance was adjusted as needed so the inoculation point was away from axillary branches). A template was used to ensure even wounding across the sample. For the 2-gallon planted seedlings, we were unsure whether to select an area of the main shoot of similar diameter to the D40 inoculation point (which would also result in less of the plant being girdled by the fungus, preserving more biomass of the plant), or continue inoculating at roughly 10 cm above the root collar. We decided to continue inoculating at 10 cm above the root collar because we could not determine another consistent reference point of inoculation that could be used. Measuring before every inoculation to inoculate at roughly the same stem diameter would have been too time consuming.

Seven-day-old plates of *C. parasitica* were used for inoculation. Using a cork borer, a 4 mm plug of the fungal mycelium was cut from the edge of the colony and fastened to the open wound of the seedling using Parafilm.

After inoculation, we observed the seedlings 3-4 days every week for 15 weeks. We were looking for the telltale signs of the seedling succumbing to the pathogen: wilting and discoloration of the leaves and death of the stem from the canker lesion up the shoot of the inoculated branch. We measured the length of the canker and reported the day on which it wilted to be used for further data analysis.

On the October 21, 2017, approximately 15 weeks after the inoculation, we observed and recorded the canker lengths of all the inoculated seedlings. Of the 1,132 inoculated seedlings, 487 plants were not successfully inoculated, meaning the fungus remained in the inoculation plug and never accepted the seedling as its growing medium, leaving 645 seedlings to extrapolate data from. This figures out to about a 57 percent inoculation accuracy. Inoculum failures ("no takes") were reported by researchers conducting similar SSA projects at other locations (Jared Westbrook, pers. comm.).

4. Results/Discussion:

4.1. Comparing the Controls

Rstudio was used for creating figures and statistical analyses (Rstudio, 2018). Student's t-test was used and determined the means of the measurement variable are statistically different between the two groups. However, while the *C. mollissima* control exhibits the predicted low canker length mean, there is abnormally high variation in canker length in the *C. dentata* control when compared to other data gathered via SSA

(Westbrook, 2018), i.e. there were some *C. dentata* individuals that had shorter canker lengths than individuals of *C. mollissima* (Figure 1). One possible explanation for this is that these trees possess some resistance to the fungus -- there are still American chestnut trees in the wild today having recovered from a blight infection, implying variation in resistance among *C. dentata* exists (Griffin et al., 1982, 1983). However, more likely is there was too much environmental noise that caused the variation in canker length in *C. dentata* to increase. This extreme variation in American chestnut canker length makes comparing the other generation types difficult.

Cross Type	Number of seeds planted	Number of D40 seedlings successfully inoculated	Average canker length at 15 week (mm)
American (AM)	**59**	**20**	**38.4**
Chinese (CH)	**43**	**19**	**17.1**
Filial Generation 1 (F1)	**61**	**32**	**36.2**
Filial Generation 2 (F2)	**31**	**13**	**47.8**
First Backcross (B1)	**258**	**86**	**48.6**
Better Backcross 1 (BB1)	**65**	**24**	**45.3**
Third Backcross Filial Generation 2 (B3F2)	**261**	**98**	**40.5**

Table 2. Seedlings planted, successfully inoculated, and the average canker length at 15 weeks by cross type

One measurement that better reflects the extreme difference in susceptibility to *C. parasitica* between control groups is percent wilted, or the percentage of individuals within a specific cross type that succumbed to the blight before the 15-week mark (where the canker lengths of the entire population were measured). We found that roughly 60 percent of the successfully inoculated American controls showed symptoms of wilting, contrasting the only one of the 19 successfully inoculated Chinese chestnut seedlings that showed these symptoms. Further analysis on days-to-wilt and percent wilting measurements gathered is required to tell if it is a reliable indicator of resistance.

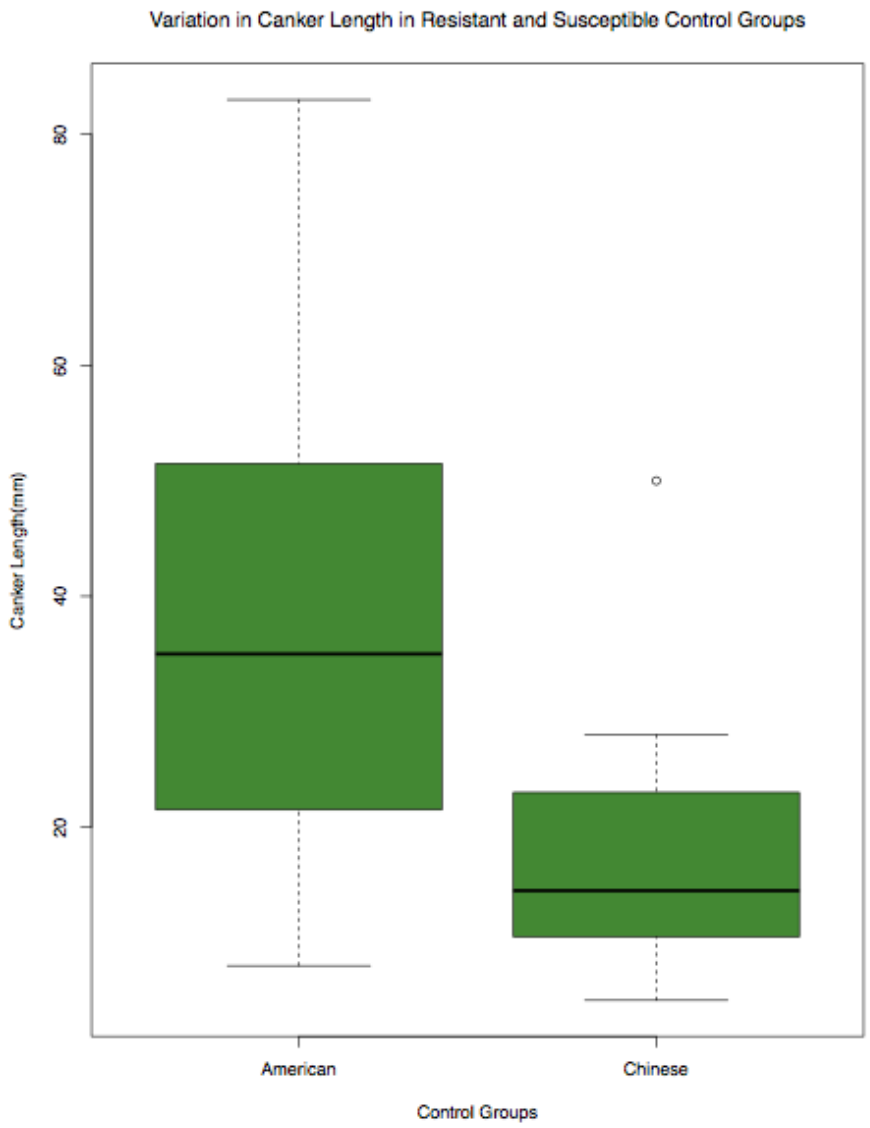

Figure 1. Blight canker lengths of susceptible *C.dentata* and resistant *C.mollissima* control group seedlings at point of inoculation. Variance between controls was significant t = 4.2907, df = 38, p-value = 5.906e-05 t-test

4.2. Variance Across Generations

After running a one-way analysis of variance and determining that canker length

differed significantly by cross type (one-way anova, $F_{6, 317}=5.856$, $P=8.3\times10^{-6}$), we used Duncan's Multiple Range Test (MRT). In Duncan's test, the difference between any two ranked means is significant if the difference exceeds a value determined by each group's standard deviation. Groups that are statistically similar are given a common letter, i.e. "a," "b," or "c," The results are shown in Table 3. The variation in canker length by generation type was plotted on a box-and-whisker chart (Figure 2.)

Treatment	Mean	Standard error	Duncan
B1	48.172	2.3436	a
BB1	45.6786	4.2711	ab
F2	45.0667	5.8355	ab
B3F2	39.9027	2.1261	b
AM	38.4	5.0537	ab
F1	36.8	3.8202	b
CH	17.1	5.0537	c

Table 3. the mean, standard error, and result of Duncan's Multiple Range Test ($P < 0.05$)

The F1 generation is heterozygous for each allele coding for blight resistance, and since blight resistance follows an incomplete dominance heritability, the mean canker length of that cross type should be about the average between the mean canker length values of the two control groups (Burnham, 1988; Steiner et al, 2016). In the F2 generation, incompletely dominant traits should exhibit a wider distribution of blight resistance when compared to the F1 generation. However, the F1 population displayed more variation in canker length than the F2 generation type, and the average canker length was approximately 9 millimeters greater than the American control. While the

B3F2 generation did yield the most resistant tree, the average of this generation also lies outside the American upper limit. The Better Backcross Generation, despite the difference in pedigree from a straight B1, did not show a significant difference from the B1 generation type.

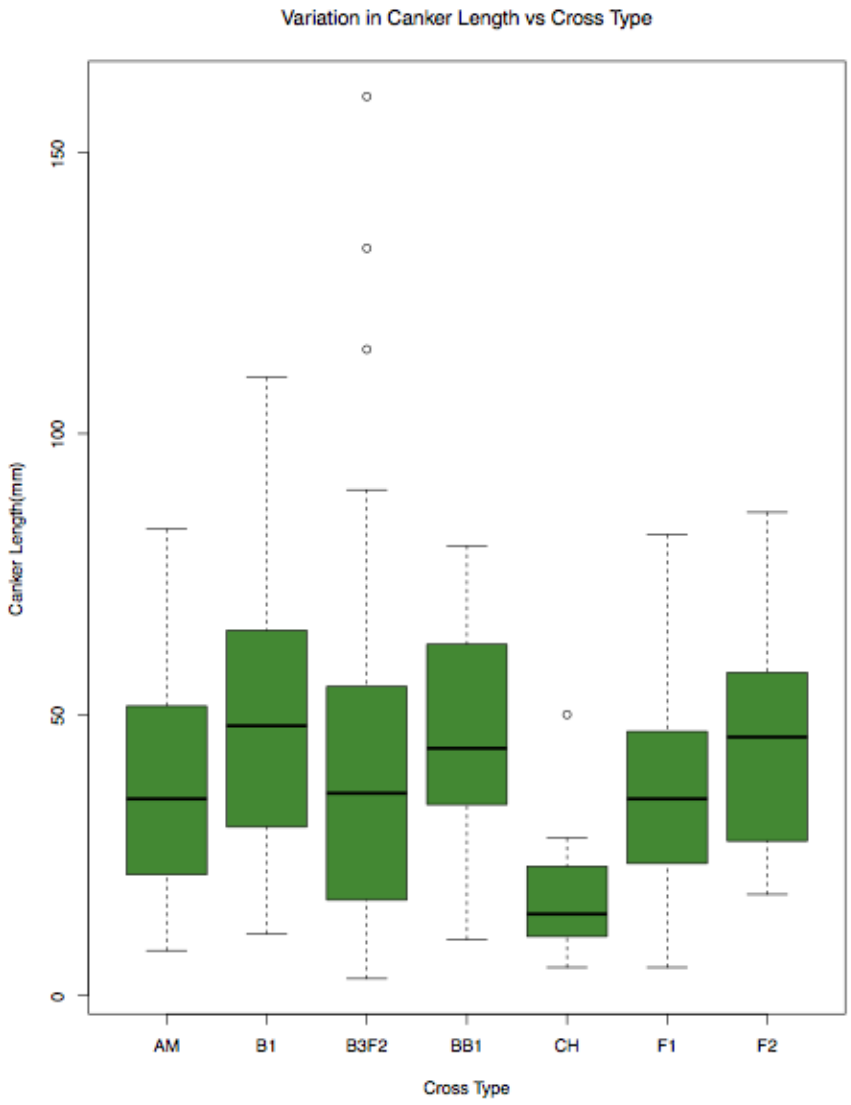

Figure 2. (American, First Backcross, Third backcross F2, Better Backcross 1, Chinese, F1, F2, respectively) Average blight canker length and variance of each cross type, including outliers. (one-way anova, $F_{6,\,317}=5.856$, $P=8.3\times10^{-6}$)

One reason for this wide variation within and among generation types may be too much environmental noise. Since less variation is observed in more mature seedlings, one way to improve on the SSA methodology is to measure cankers at 24 weeks. Jared Westbrook saw the greatest difference in canker length between American and Chinese controls 24 weeks post inoculation (Westbrook, 2018). As for the low rates of successful inoculations, this is a problem that has seen a great amount of variance across TACF in 2018. Some experimenters reported 100 percent successful inoculations, others saw little/no successful inoculations, leading TACF to work with different strains of *C. parasitica* to address this issue (Ben Jarret, Pers. Comm.).

Average canker lengths by family will be analyzed by Margaret Miller as part of her Masters Thesis project at a later date. Given the inconsistent results based on canker type, I predict there will be no significant variation between family types of the same generation type.

Too much environmental noise exists in order to adequately differentiate between seedlings of different generation types. Although the American and Chinese controls did exhibit statistically significant differences in blight resistance to chestnut blight, there was too much variation in the American control to effectively compare its resistance to other hybrid crosses. Although resistance to chestnut blight follows an incompletely dominant pattern of inheritance, the heterozygous F1 cross type mean canker length was not the average of the two control groups. The F2 and B3F2 segregating populations did not exhibit the highest variation in canker length as hypothesized. Many of the interspecific hybrid crosses didn't show an average canker length in between the two

controls. These observations paired with data collected by other members of TACF from standard assays done on 3-5-year-old trees suggest the small stem assay has low resolution in determining blight resistance.

5. References

Anagnostakis, S.L., (2001). The effect of multiple importations of pests and pathogens on a native tree. Biological Invasions 3: 245–254

Anagnostakis, S.L. (1987). Chestnut Blight: The Classical Problem of an Inducted Pathogen. *Mycologia* 79(1): 23-37

Anagnostakis, S.L. (1977). Vegetative Incompatibility in *Endothia parasitica.* *Experimental Mycology* 1: 306-316

Anderson PJ. (1914). The morphology and life history of the chestnut blight fungus. *Pennsylvania Chestnut Tree Blight Commission* 7: 3-44

Burnham, C.R. (1988). The Restoration of the American Chestnut: Mendelian genetics may solve a problem that has resisted other approaches. *American Scientist* 76: 478-487

Burnham, C.R. (1987). Backcross Breeding: Blight-Resistant American Chestnuts II. Hardier Chinese Chestnuts. *The Journal of The American Chestnut Foundation* 2(1): 12-17

Burnham, C.R. (1981). Blight-resistant American Chestnut: There's Hope. *Plant Disease* 65: 459–460

Faison, E. K., and Foster, D. R. (2014). Did American Chestnut Really Dominate the

Eastern Forest? Arnoldia 72 (2): 18-32

Fang, G. C., Blackmon, B. P., Staton, M. E., Nelson, C. D., Kubisiak, T. L., Olukolu, B. A., ... & Monsanto, M. (2013). A physical map of the Chinese chestnut (Castanea mollissima) genome and its integration with the genetic map. *Tree genetics & genomes* 9(2): 525-537.

Griffin, G. J., Hebard, F. V., Wendt, R. W., Elkins, J. R. (1983). Survival of American chestnut trees: evaluation of blight resistance and virulence in Endothia parasitica. *Phytopathology* 73: 1084–1092

Griffin, G. J., F. V. Hebard, and J. R. Elkins. (1982). Blight resistance in American chestnut. *73rd An. Rpt. Northern Nut Growers Assn*. pp. 66-73.

Harlan H.V., Pope M.N. (1922). The use and value of back-crosses in small-grain breeding. J Hered 13:319–322

Hebard F.V. (2006). The Backcross Breeding Program of the American Chestnut Foundation. *Journal of The American Chestnut Foundation* 19: 55–77

Jaynes, R. A. (1962). Chestnut chromosomes. *For. Sci*. 8:372-77.

Kubisiak, T. L., Hebard, F. V., Nelson, C. D., Zhang, J., Bernatzky, R., Huang, H., Anagnostakis, S. L., and Doudrick, R. L. (1997). Molecular mapping of resistance to blight in an interspecific cross in the genus Castanea. *Phytopathology* 87: 751-759

Merkel, H. W. (1905). A deadly fungus on the American chestnut. *New York Zool. Soc.* 10. *Ann. Report* 10: 97-103

RStudio Team (2018). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Russell, Emily W.B., 1987. Pre-Blight Distribution of Castanea dentata (Marsh.) Borkh. *Bulletin of the Torrey Botanical Club*, 114 (2): 183-190

Santos, C., Nelson, C., Zhebentyayeva, T., Machado, H., Gomes-Laranjo, J., Costa, R. (2017). First interspecific genetic linkage map for Castanea sativa x Castanea crenata revealed QTLs for resistance to Phytophthora cinnamomi. *PLoS ONE* 12(9): e0184381.

Stevens, R.B. (1960). *Plant Pathology, an Advanced Treatise* 3: 357-429

Steiner, K.C., Westbrook, J.W., Hebard, F.V., Georgi, L.L., Powell, W.A., Fitzsimmons, S.F. (2016). Rescue of the American chestnut with extraspecific genes following its destruction by a naturalized pathogen. *New Forests* DOI 10.11007/s11056-016-9561-5

Westbrook, J. and Jarret, B. (2018). Accelerating blight resistance screening. *The Journal of the American Chestnut Foundation* 32(1): 22-24